Bioprospecting Endophytic Fungi Cladosporium genus as Source of Bioactive Metabolites

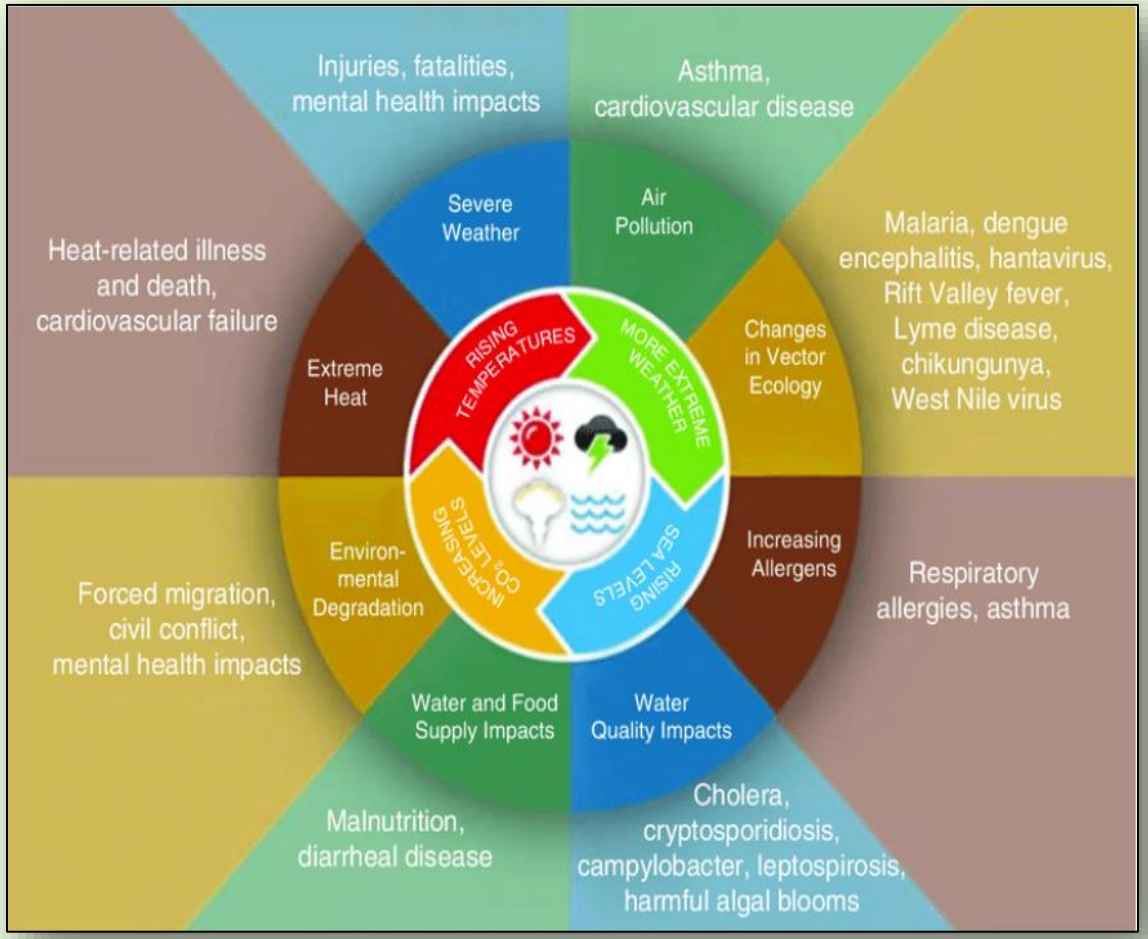
Ruby Bhullar Garcha
(Independent Researcher)

Learning Objectives:

- World Health Problems to Sustainable Development Goals
- Endophytes

a. Fungal Endophytes – rich source of functional metabolites
b. Why and How to Bio-prospect aspect of Endophytes
c. Different approaches applied to Un-culturable and Culturable microbes
d. Diverse domains out of which fungal endophytes have emerged as an important source of bioactive compounds
e. Diverse species of fungal endophytes highlighting Cladosporium genus
f. Case Study of Cladosporium isolates
g. Fungal Endophytes to Agricultural productivity
h. Future Perspectives to Research

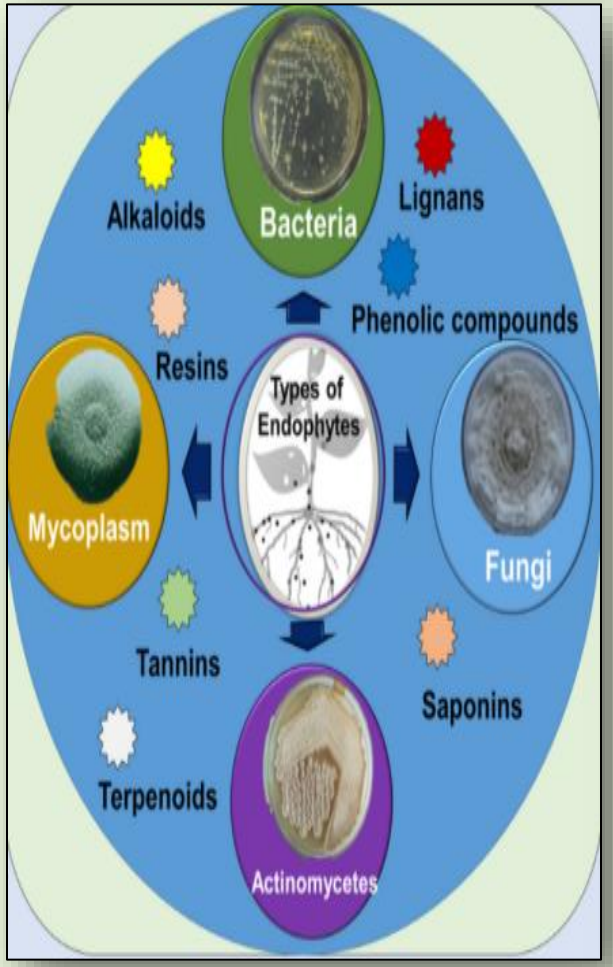
From Global Health Problems to Sustainable Development Goals:

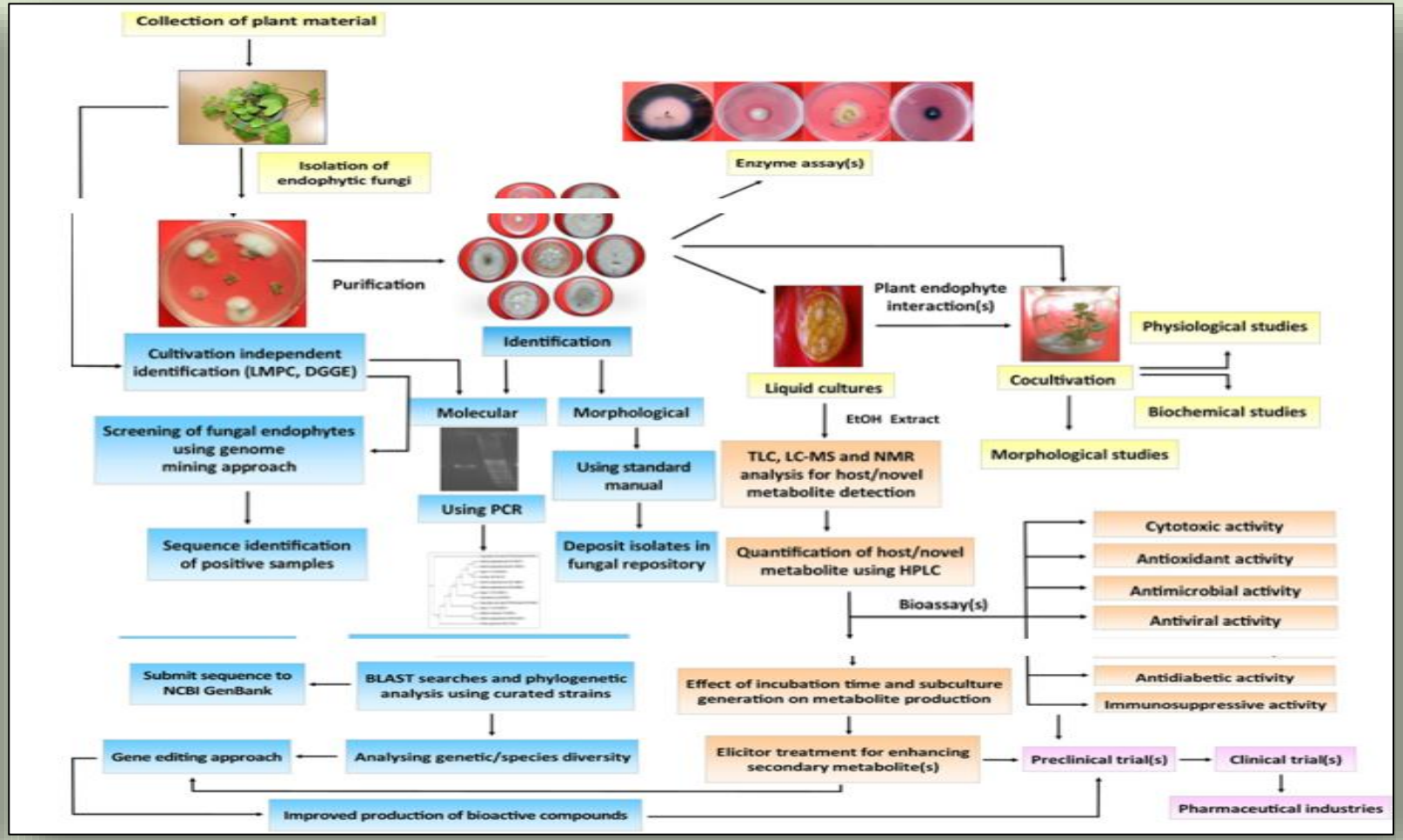




DOI:10.1007/978-3-319-96920-

Endophytes as a novel and rich source of functional metabolites:

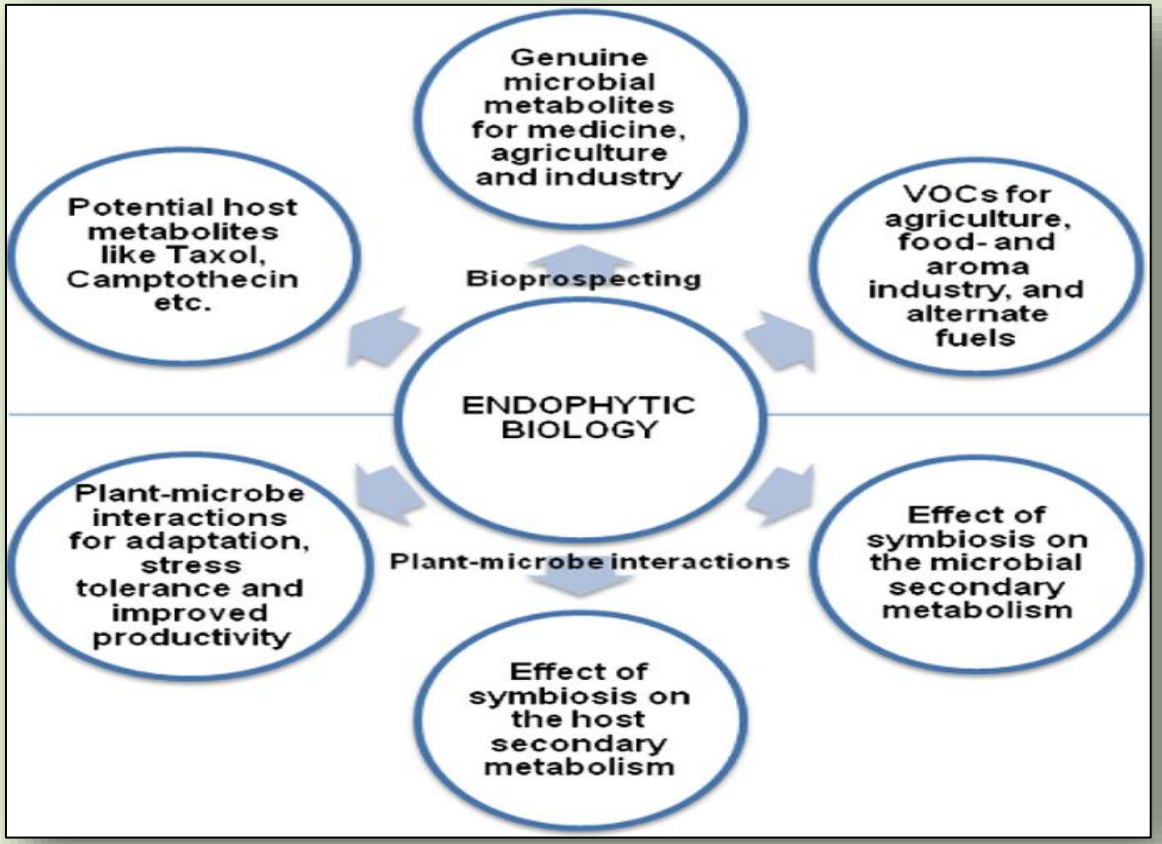


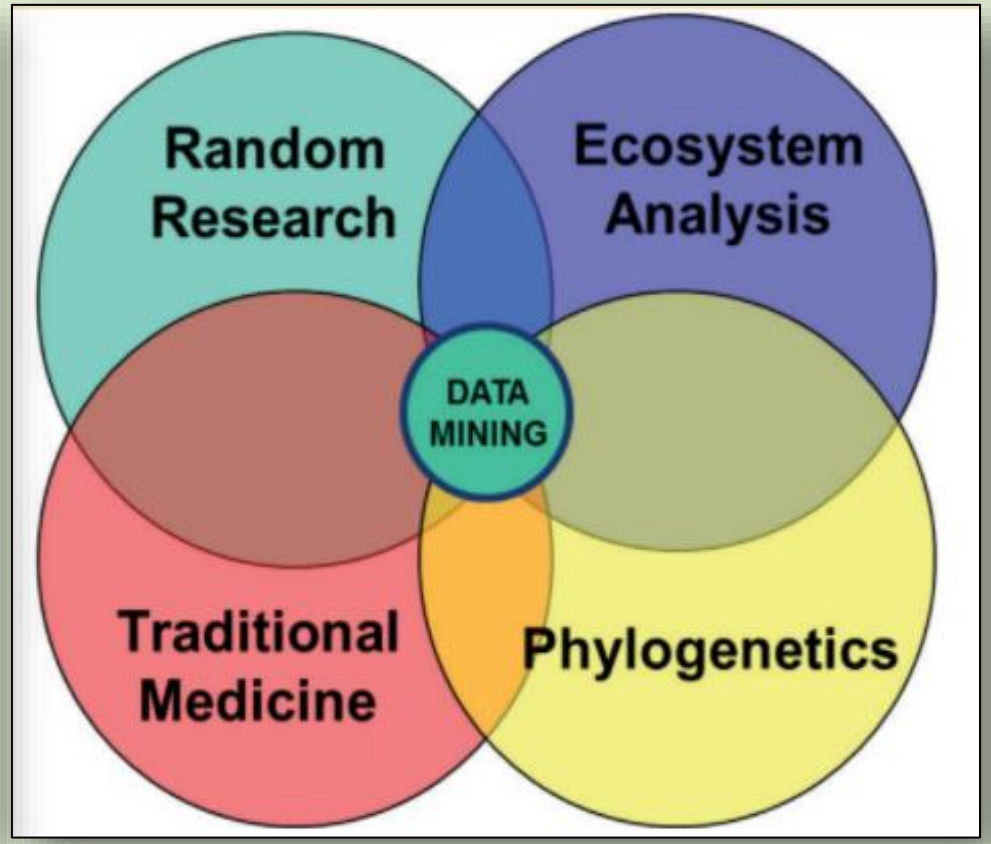


Front. Microbiol., 2016 | https://doi.org/10.3389/fmicb.2016.01538

https://doi.org/10.1016/j.biotechadv.2019.107462

Bioprospecting Endophytes:

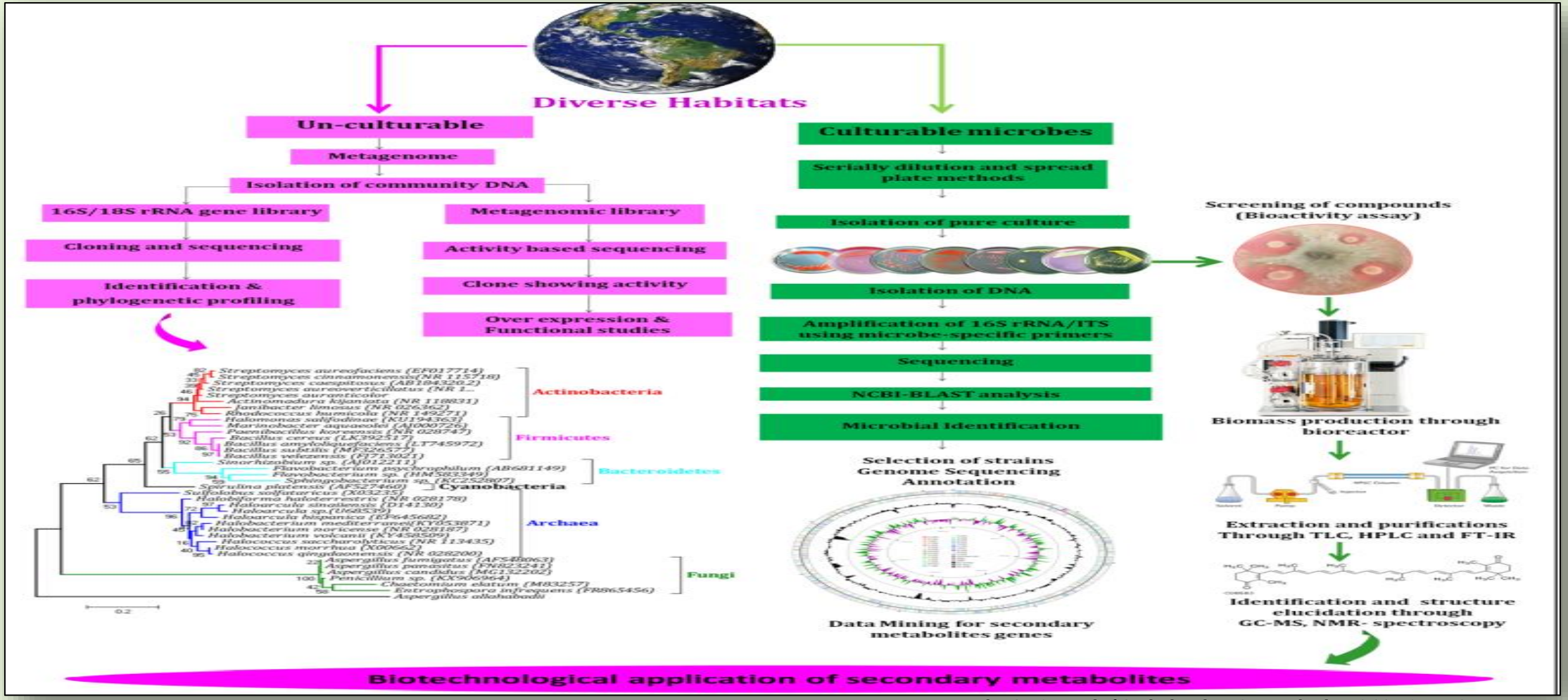




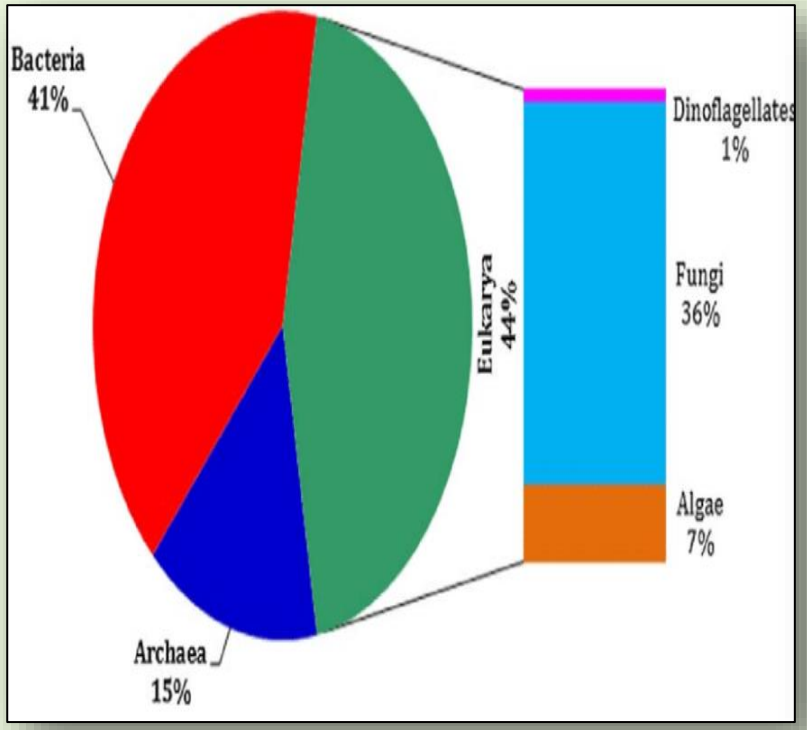
DOI:10.1007/s00253-015-6487-3 Corpus ID: 17033286

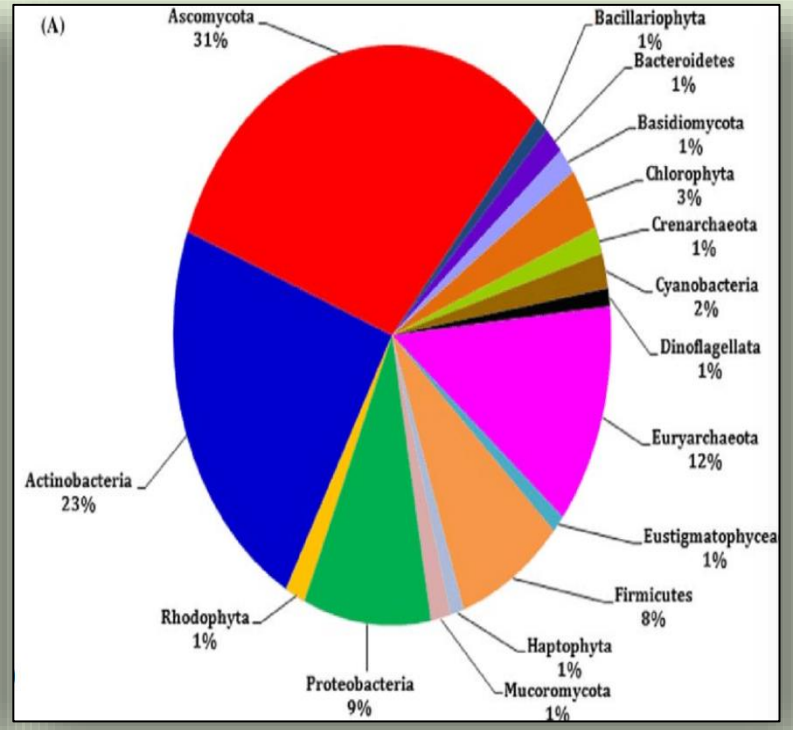
Metabolomics of Endophytic Fungi Producing Associated Plant Secondary Metabolites: Progress, Challenges and Opportunities

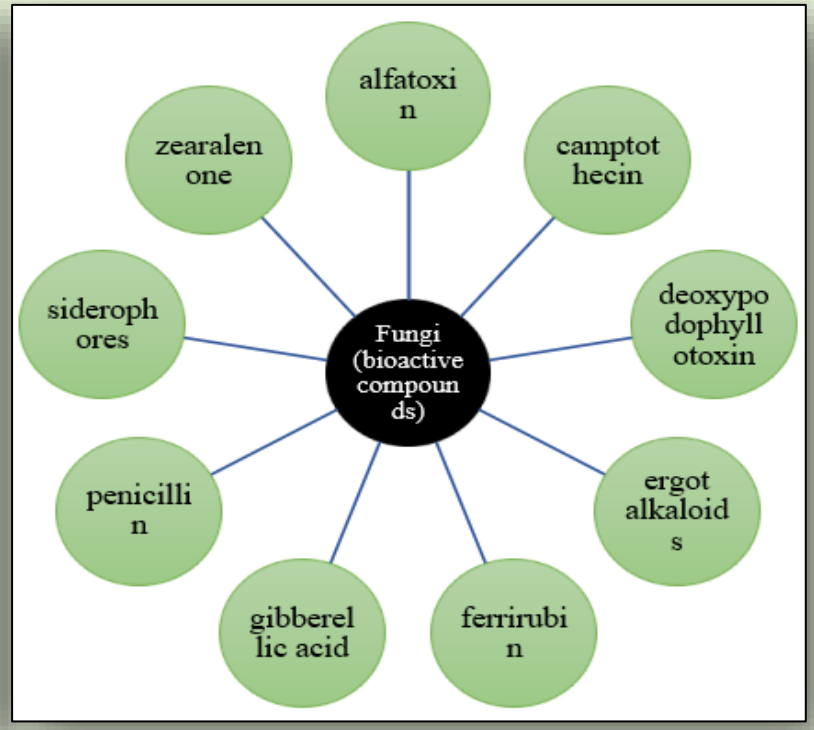
Methods Applied on Microbial Communities:



Diverse Domains and Phyla:

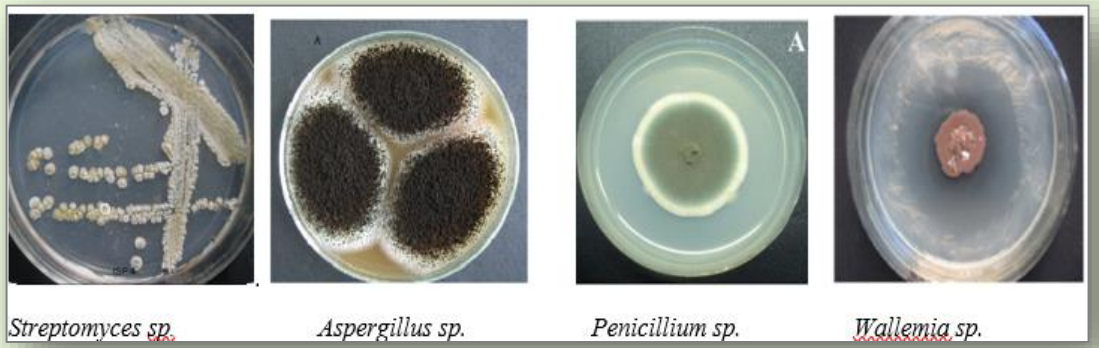


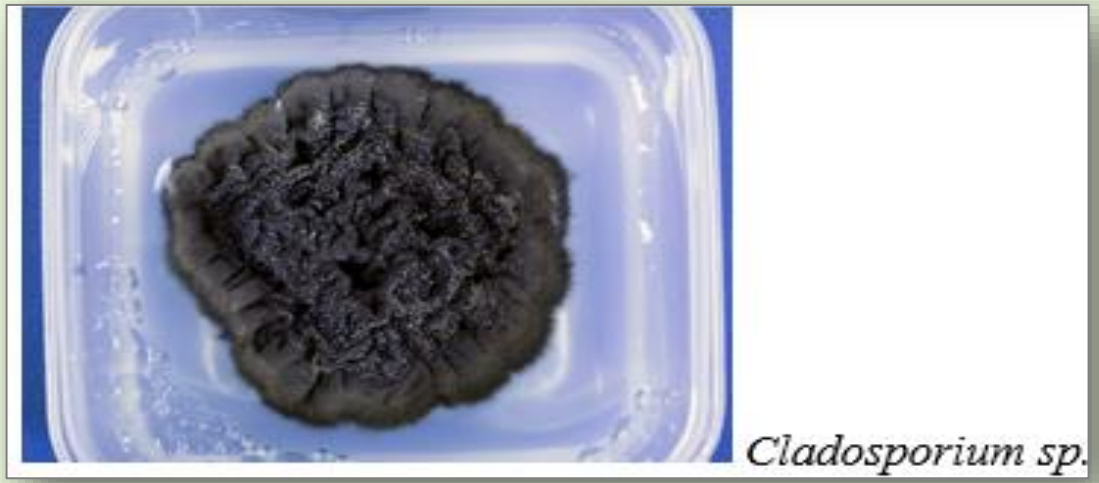


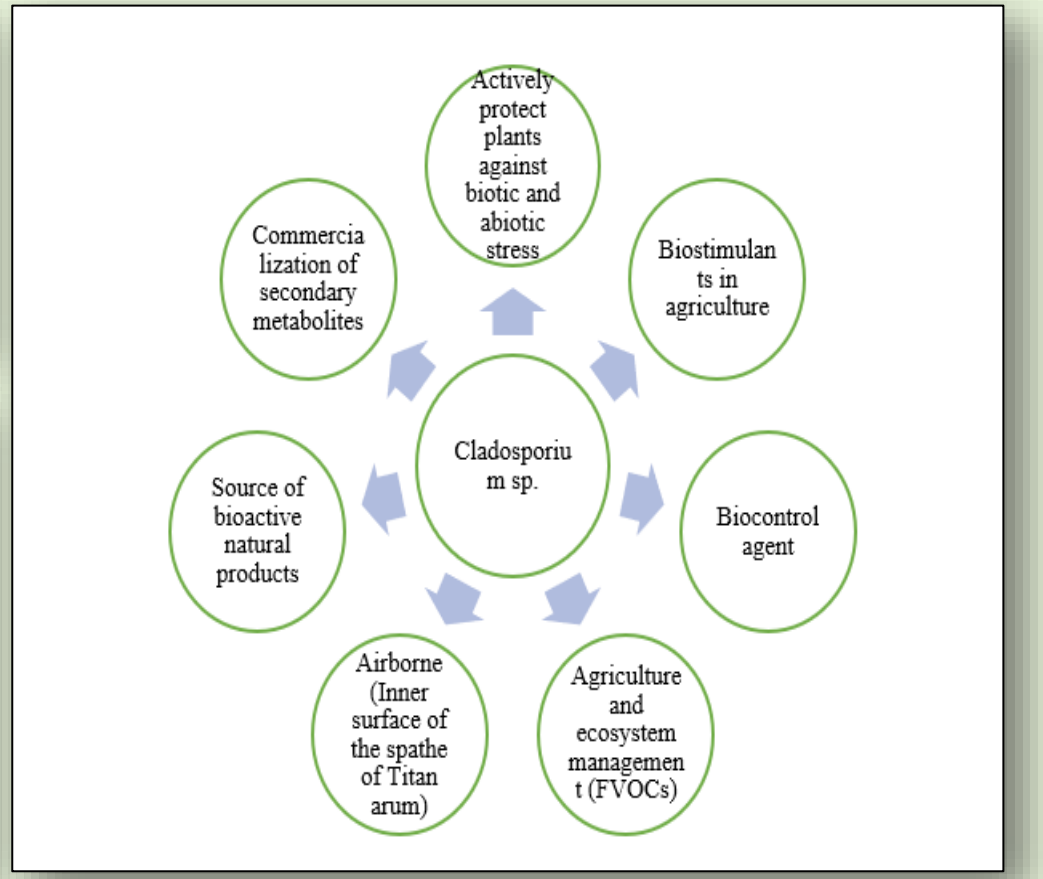


New and Future Developments in Microbial Biotechnology and Bioengineering. DOI: https://doi.org/10.1016/B978-0-444-63504-4.00020-7

Diverse *Species* of Fungal Endophytes:

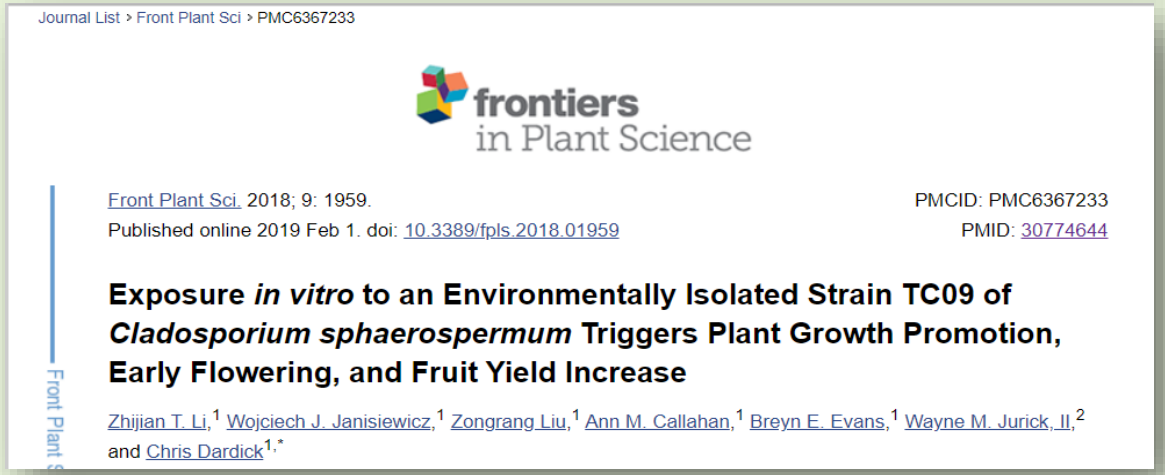






Lugtenberg, B., Caradus, J., Johnson, L. 2016 Fungal Endophytes for Sustainable Crop Production

What draws our attention to *Cladosporium sp.*



Journal List › Front Plant Sci › PMC6367233

frontiers in Plant Science

Front Plant Sci. 2018; 9: 1959.
Published online 2019 Feb 1. doi: 10.3389/fpls.2018.01959

PMCID: PMC6367233
PMID: 30774644

Exposure *in vitro* to an Environmentally Isolated Strain TC09 of *Cladosporium sphaerospermum* Triggers Plant Growth Promotion, Early Flowering, and Fruit Yield Increase

Zhijian T. Li,[1] Wojciech J. Janisiewicz,[1] Zongrang Liu,[1] Ann M. Callahan,[1] Breyn E. Evans,[1] Wayne M. Jurick, II,[2] and Chris Dardick[1,*]

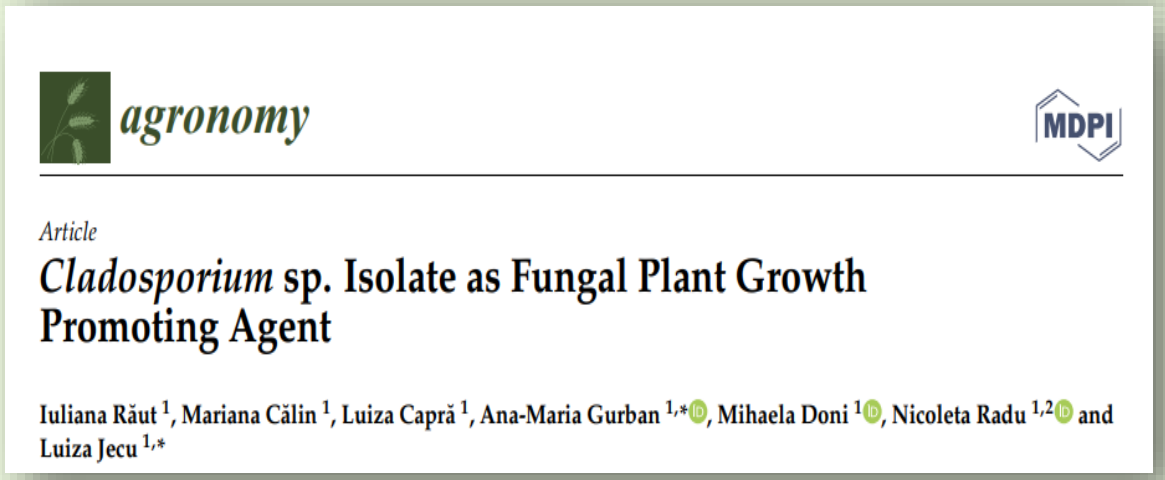


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Search A

Terrestrial Fungus May Be Key To Farming In Space

Terrestrial Fungus May Be Key To Farming In Space



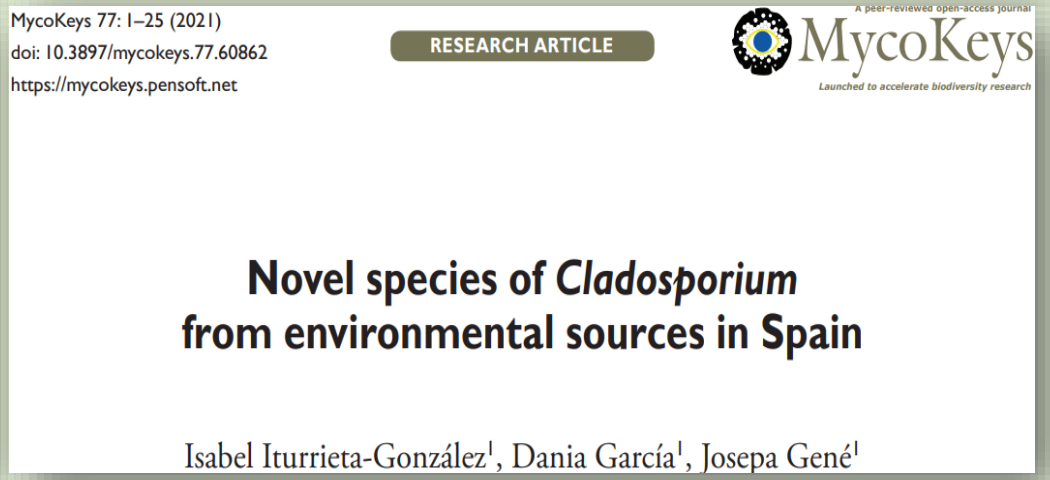
agronomy

MDPI

Article

Cladosporium sp. Isolate as Fungal Plant Growth Promoting Agent

Iuliana Răut [1], Mariana Călin [1], Luiza Capră [1], Ana-Maria Gurban [1,*], Mihaela Doni [1], Nicoleta Radu [1,2] and Luiza Jecu [1,*]



MycoKeys 77: 1–25 (2021)
doi: 10.3897/mycokeys.77.60862
https://mycokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
MycoKeys
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Novel species of *Cladosporium* from environmental sources in Spain

Isabel Iturrieta-González[1], Dania García[1], Josepa Gené[1]

Case Study of *Cladosporium* isolates:

agronomy

MDPI

Article

Cladosporium sp. Isolate as Fungal Plant Growth Promoting Agent

Iuliana Răut [1], Mariana Călin [1], Luiza Capră [1], Ana-Maria Gurban [1,*], Mihaela Doni [1], Nicoleta Radu [1,2] and Luiza Jecu [1,*]

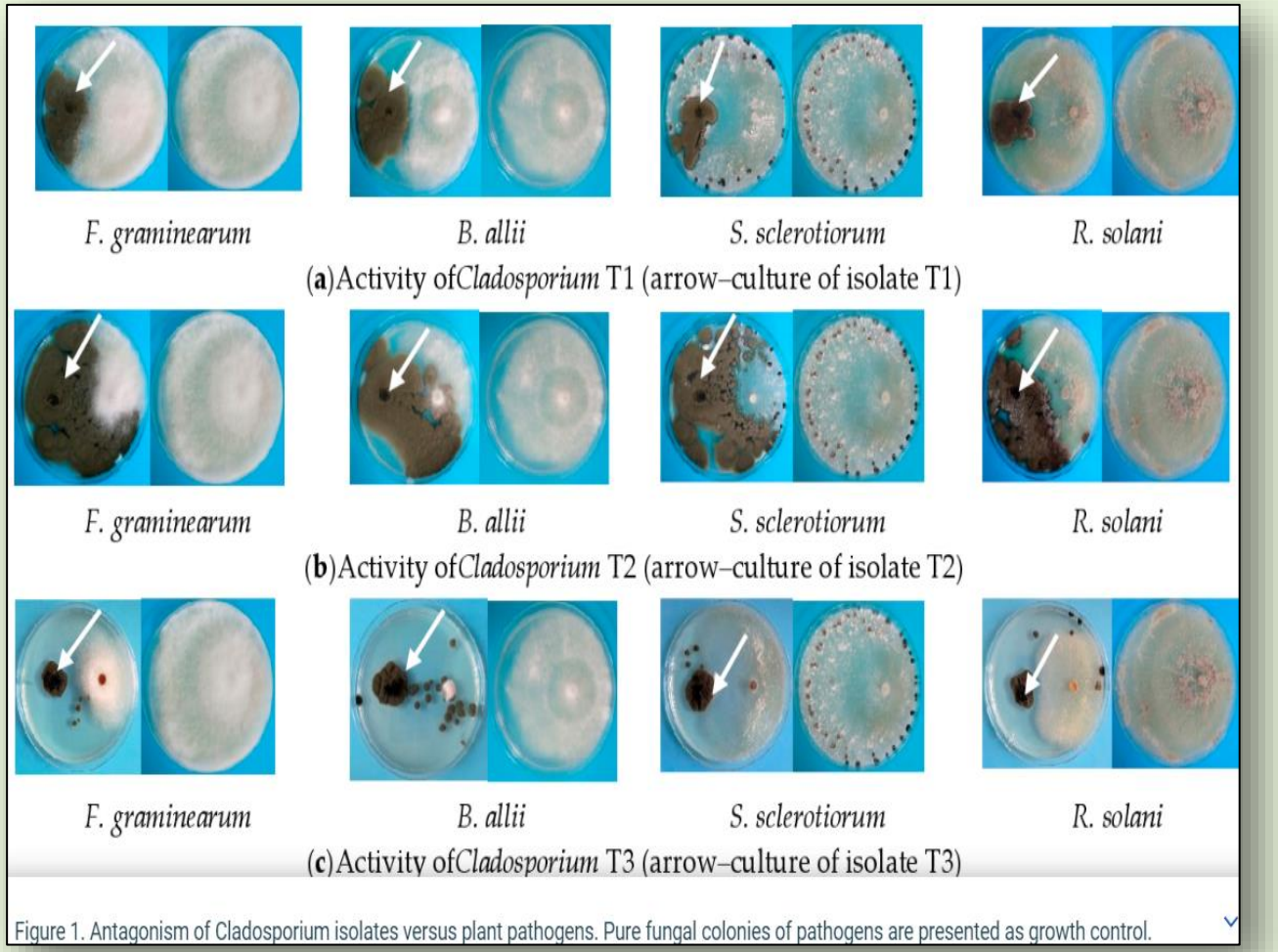


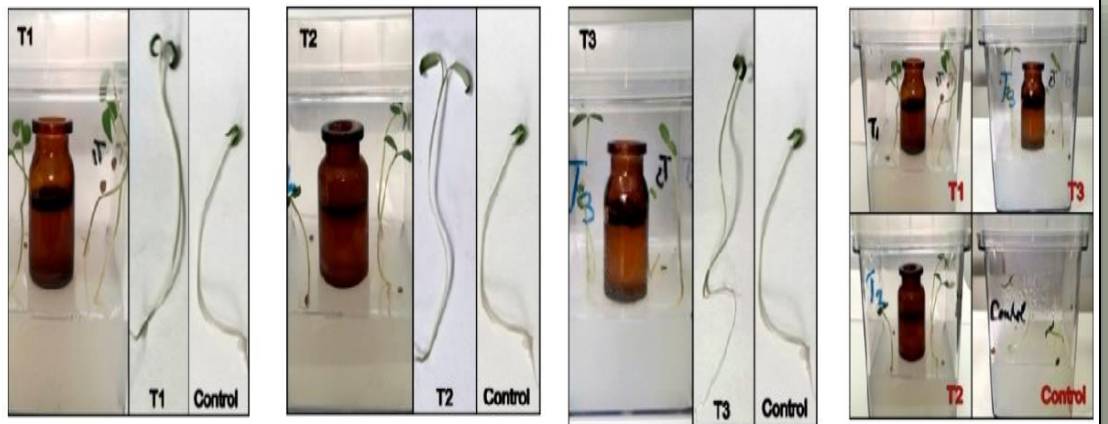
Figure 1. Antagonism of Cladosporium isolates versus plant pathogens. Pure fungal colonies of pathogens are presented as growth control.

Antagonism vs Plant pathogens and Effect of Volatiles:

Antagonistic Fungal Isolate	Inhibition * (%)			
	F. graminearum	*B. allii*	*S. sclerotiorum*	*R. solani*
Cladosporium sp. T1	35.6 ± 0.5	37.1 ± 0.4	40.8 ± 0.9	35.5 ± 0.5
Cladosporium sp. T2	**57.4 ± 0.5**	86.4 ± 0.5	**62.6 ± 0.5**	**56.9 ± 0.9**
Cladosporium sp. T3	47.1 ± 0.4	58.3 ± 0.6	29.2 ± 0.4	41.7 ± 0.5

* Values are the average of three independent experiments ± standard deviations.

Table 1. Inhibition of pathogens by Cladosporium isolates.



Exposure toT1 isolate Exposure toT2 isolate Exposure toT3 isolate Exposure toall isolates

Figure 2. Growth promotion of tomato seedling by exposure to volatiles secreted by Cladosporium isolates. The photographs were taken 21 days after seed sowing.

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Plant height compared to Control and Secretion of Hydrolytic Enzymes:

Fungal Isolate	Growth Parameters *				
	Total Weight (g)	Total Height (cm)	Plant Height (cm)	Root System Length (cm)	Number of Leaves
Cladosporium sp. T1	0.06 ± 0.01	10.0 ± 0.3	7.5 ± 0.2	2.5 ± 0.2	Two leaves of medium size
Cladosporium sp. T2	0.06 ± 0.01	**10.5 ± 0.2**	**7.5 ± 0.4**	**3.5 ± 0.3**	Two leaves of medium size
Cladosporium sp. T3	0.06 ± 0.01	10.3 ± 0.3	6.8 ± 0.4	3.2 ± 0.2	Two leaves of medium size
Control	0.03 ± 0.01	7.5 ± 0.2	5.5 ± 0.2	2.0 ± 0.3	Two leaves of small size

* Values are the average of three independent experiments ± standard deviations.

Table 2. Plant growth parameters after vo atiles exposure.

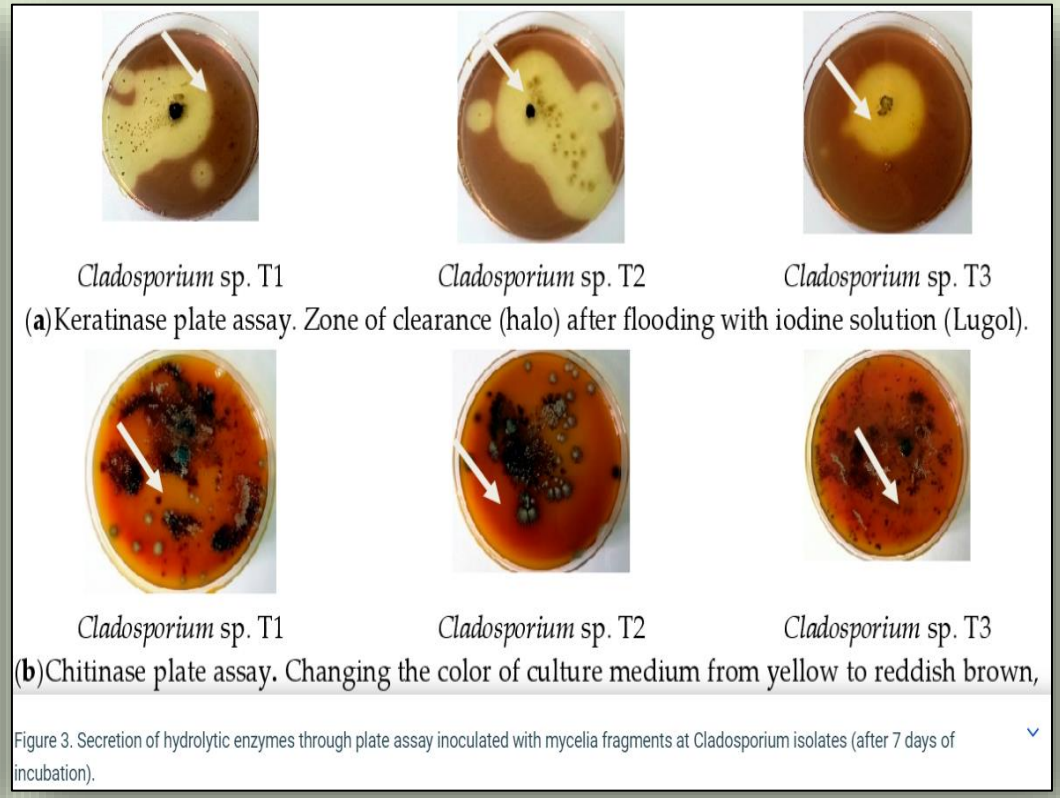


Figure 3. Secretion of hydrolytic enzymes through plate assay inoculated with mycelia fragments at Cladosporium isolates (after 7 days of incubation).

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Cladosporium isolate T2 grown on feathers medium exhibited highest value of Keratinase, Chitinase, and Cellulase Activity:

Culture Filtrate from Isolates	Colony Diameter (cm) *		
	Keratinase Activity	*Chitinase* Activity	*Cellulase* Activity
Cladosporium sp. T1 (medium without feathers)	0	0	0.6 ± 0.05 mm
Cladosporium sp. T1 (medium with feathers)	2.6 ± 0.05	2.2 ± 0.1	2.2 ± 0.05
Cladosporium sp. T2 (medium without feathers)	0.7 ± 0.05	0.7 ± 0.1	0.6 ± 0.05
Cladosporium sp. T2 (medium with feathers)	**4.3 ± 0.05**	**3.4 ± 0.09**	**3.1 ± 0.08**
Cladosporium sp. T3 (medium without feathers)	0.6 ± 0.06	0.8 ± 0.05	0.8 ± 0.08
Cladosporium sp. T3 (medium with feathers)	3.2 ± 0.00	3.2 ± 0.05	2.4 ± 0.09

Table 3. Colony diameter in plate assay inoculated with culture filtrates for detection of hydrolytic enzymes.

* Values are the average of three independent experiments ± standard deviations.

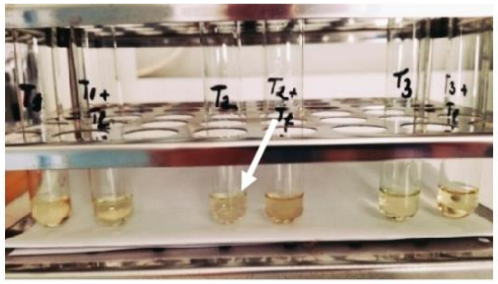
No keratinase and chitinase activities were obtained from *Cladosporium* isolate T1. As expected, the enzymatic activities exhibited by fungal isolates were higher in the

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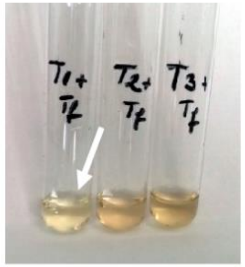
Cladosporium sp. Isolate as Fungal Plant Growth Promoting Agent

I. Răut, M. Călin, +4 authors L. Jecu

IAA production by fungal isolates; Zinc and Phosphorus Solubilisation:



(**a**) Colorimetric test for IAA production



(**b**) PDB medium + L-tryptophan

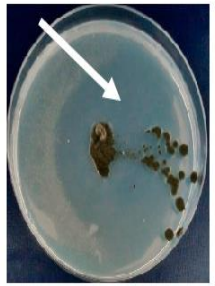
Figure 4. Images of test for IAA production at fungal isolates (T1, T2, T3—supernatants from fungal cultivation on PDB medium; T1 + Tf, T2 + Tf, T3 + Tf—supernatants from fungal cultivation on PDB medium supplemented with L-tryptophan; arrow—pink color). Collapse

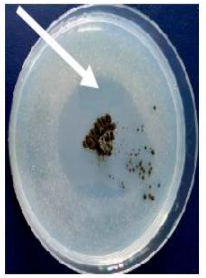
Strain	DO* 530 nm (PDB Medium)	DO* 530 nm (PDB + L-Tryptophan)
Cladosporium T1	0.014 ± 0.001	0.024 ± 0.001
Cladosporium T2	**0.076 ± 0.002**	**0.125 ± 0.012**
Cladosporium T3	0.047 ± 0.001	0.102 ± 0.002
Control (culture medium)	0.010 ±0.002	0.014 ± 0.001

* Values are the average of three independent experiments ± standard deviations.

Table 4. Production of IAA by fungal isolates on culture media.

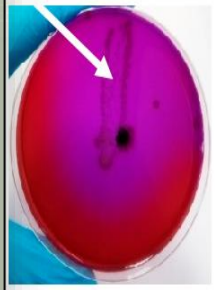




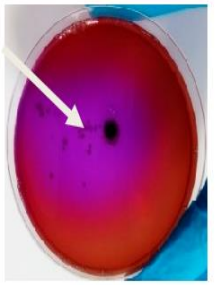


Cladosporium sp. T1 *Cladosporium* sp.T2 *Cladosporium* sp.T3

(**a**)Zn (as 0.1% ZnO) solubilization. Appearance of clear zone (halo) around mycelium, as Zn solubilization







Cladosporium sp. T1 *Cladosporium* sp.T2 *Cladosporium* sp.T3

Figure 5. Images of zinc and phosphorus solubilization at Cladosporium isolates (images after seven days of incubation).

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Cladosporium sp. Isolate as Fungal Plant Growth Promoting Agent

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Effect on tomato growth exposed to *Cladosporium* isolates:

Sample	Total Nitrogen * (Kjeldhal) (mg/mg)		Ammonium Nitrogen * (mg/mg)		Protein Nitrogen * (mg/mg)	
	Medium no Feathers	Medium with Feathers	Medium no Feathers	Medium with Feathers	Medium no Feathers	Medium with Feathers
Cladosporium T1 isolate	127×10^{-3}	137×10^{-3}	61×10^{-3}	87×10^{-3}	66×10^{-3}	50×10^{-3}
Cladosporium T2 isolate	90×10^{-3}	**569×10^{-3}**	93×10^{-3}	**211×10^{-3}**	55×10^{-3}	**358×10^{-3}**
Cladosporium T3 isolate	102×10^{-3}	120×10^{-3}	88×10^{-3}	87×10^{-3}	33×10^{-3}	nd
Minimal basal medium-Control	80×10^{-3}	96×10^{-3}	60×10^{-3}	84×10^{-3}	20×10^{-3}	12×10^{-3}

(*) Total nitrogen–Kjeldhal method; ammonium nitrogen calculated according to standard SR EN 15475:2009; protein nitrogen–calculated as total nitrogen minus ammonium nitrogen.

Table 5. Nitrogen content of protein hydrolysates obtained from cultivation of Cladosporium isolates in experimental conditions (*).



(**a**) Plants exposed to PHs from isolates cultured on medium *without feathers*



(**b**) Plants exposed to PHs from isolates cultured on medium *with feathers*



(**c**) Plants collected after 1 month of treatment with PHs from isolates grown on medium *without feathers*



(**d**) Plants collected after 1 month of treatment with PHs from isolates grown on medium *with feathers*

Figure 6. Images of tomato plants treated with protein hydrolysates from Cladosporium isolates (fungal isolates T1, T2, and T3 cultured on medium without feathers; fungal isolates T1P, T2P and T3P cultured on medium with feathers; C—Control, treatment with tap water). Collapse

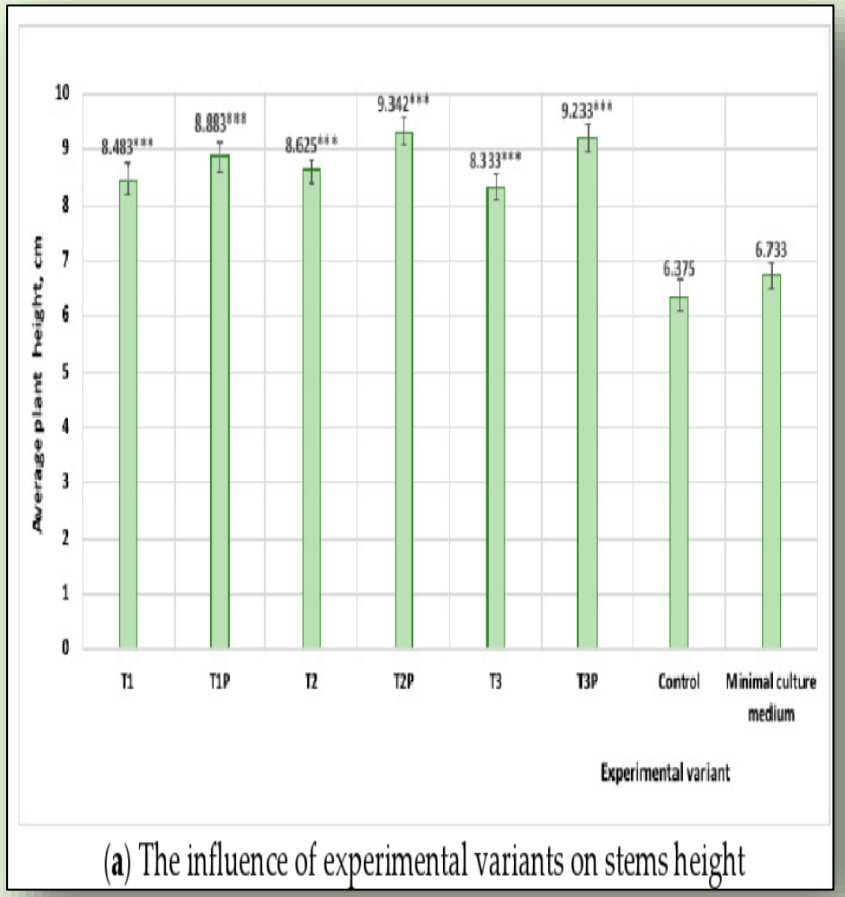
Table 6. Effects on tomato growth exposed to treatment with protein hydrolysates from *Cladosporium* isolates cultured on medium with or without feathers.

Protein Hydrolysates Obtained from *Cladosporium* Cultures Grown on Different Media	Stem Height * (cm)	Stem Weight * (g)	Root Weight * (g)
Isolate T1 (medium without feathers)	8.48 ± 0.9	0.49 ± 0.09	0.06 ± 0.01
Isolate T1P (medium with feathers)	8.88 ± 0.9	0.48 ± 0.09	0.09 ± 0.02
Isolate T2 (medium without feathers)	8.62 ± 0.9	0.55± 0.08	0.07 ± 0.01
Isolate T2P (medium with feathers)	**9.34 ± 0.7**	**0.65 ± 0.1**	**0.09 ± 0.03**
Isolate T3 (medium without feathers)	8.33 ± 0.7	0.44 ± 0.08	0.07 ± 0.01
Isolate T3P (medium with feathers)	9.23 ± 0.9	0.51 ± 0.08	0.09 ± 0.03
Minimal medium (MM)	6.73 ± 0.8	0.38 ± 0.07	0.06 ± 0.02
Control (water)	6.37 ± 0.9	0.23 ± 0.07	0.04 ± 0.02

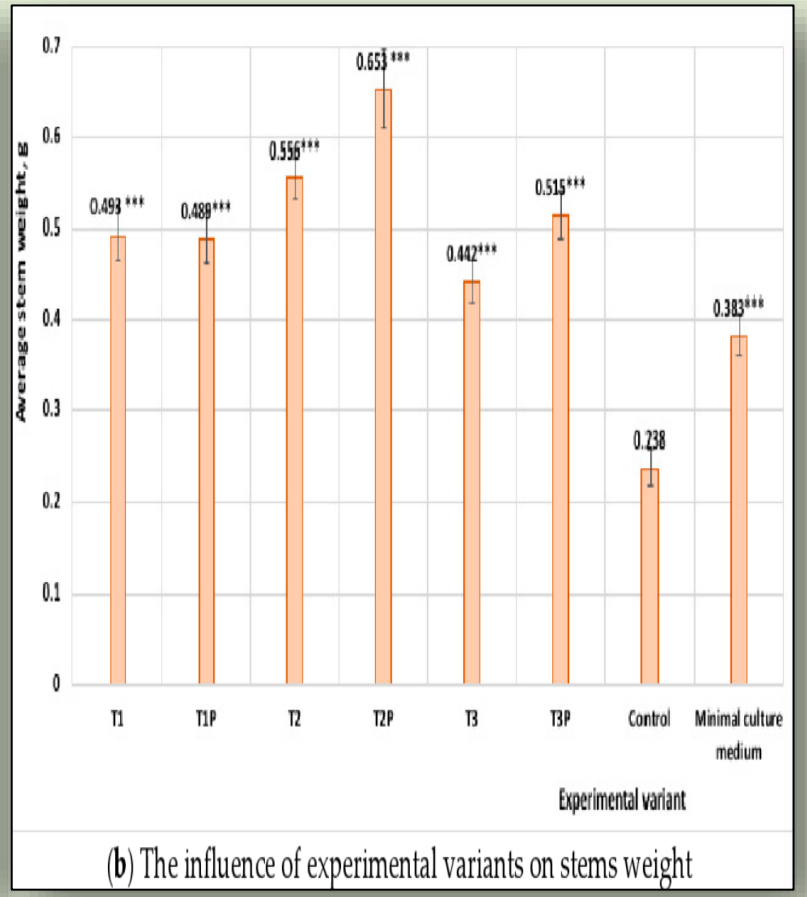
* Values are the average of measurements of 12 plants randomly selected from multicellular trays ± standard deviations.

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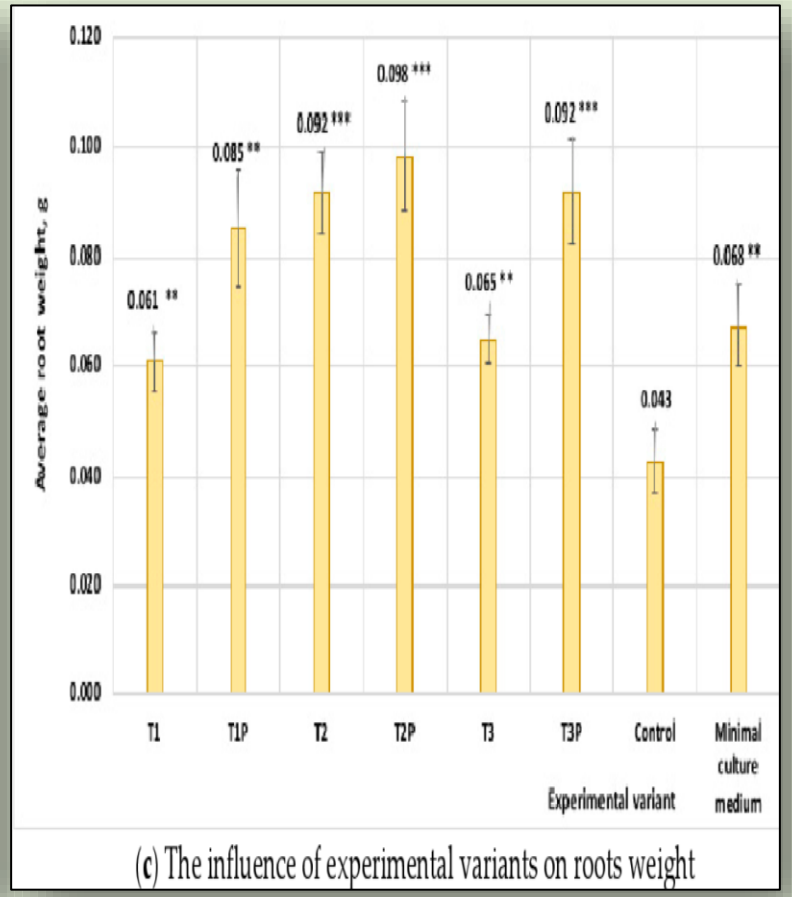
Statistic Analysis about characteristics of Cladosporium isolates:



(a) The influence of experimental variants on stems height



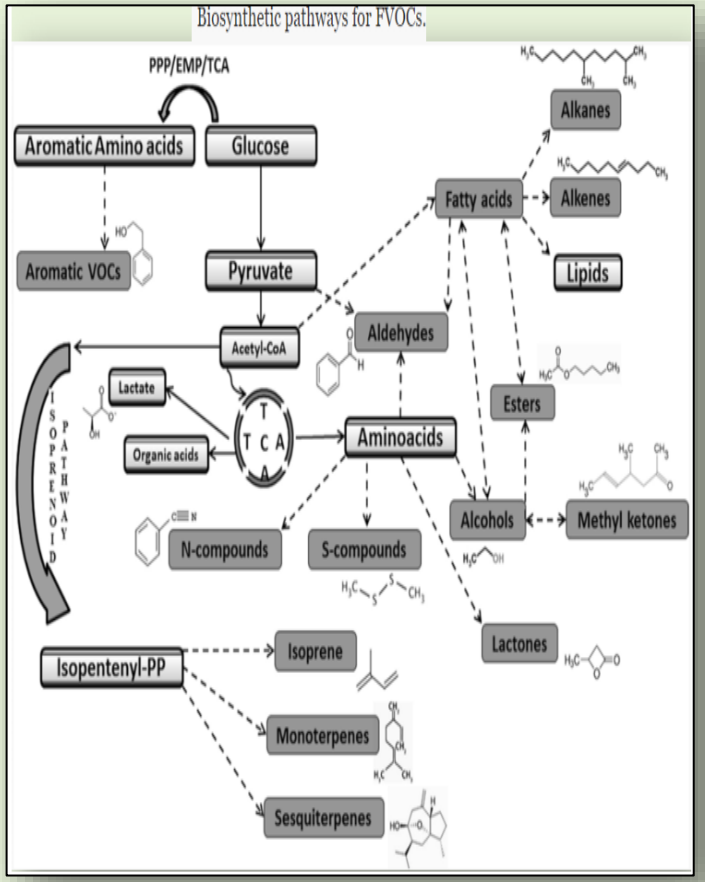
(b) The influence of experimental variants on stems weight



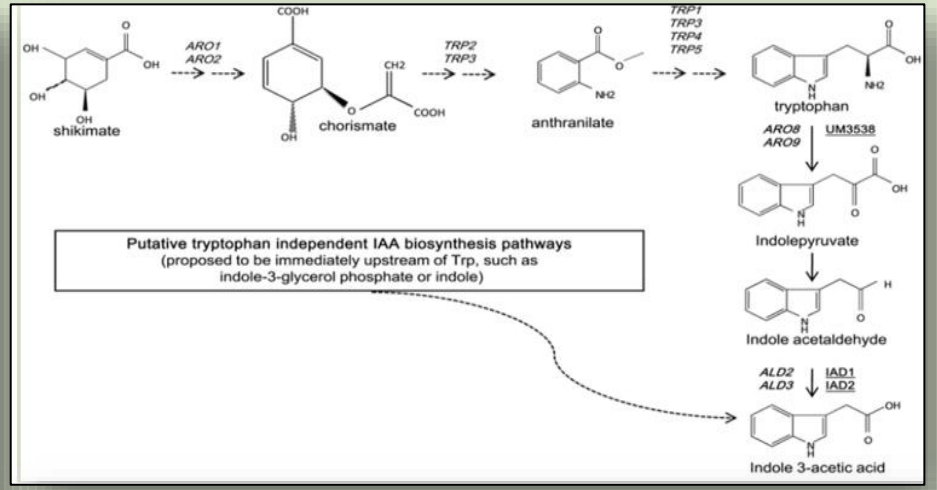
(c) The influence of experimental variants on roots weight

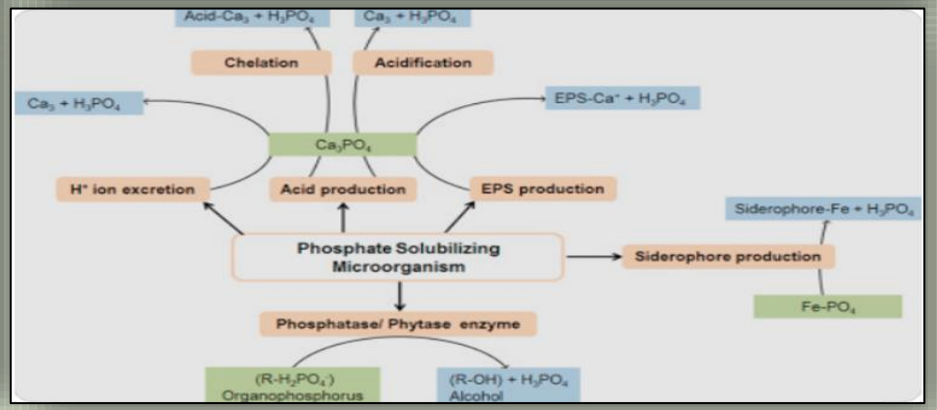
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Fungal Endophytes to Agricultural Productivity:



Agricultural sector	Growth promotion
	Biotic and Abiotic stress
	Phytohormones
	Antimicrobial compounds
	Agrochemical bioactive metabolites
	Safe and cost effective alternative to chemical pesticides and fertilizers
	Commercializing antagonistic capability as biocontrol agents
Industrial Sector	Fungal enzymes
	Research on enzymatic capability
	Hydrolytic and oxidative extracellular enzymes
	Bioremediation and Biotransformation





Springer, Cham. https://doi.org/10.1007/978-3-030-14846-1_5

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DOI:10.1534/genetics.109.112854

https://doi.org/10.1016/B978-0-12-817497-5.00011-2

Conclusion and Future Perspectives:

- The fungi belonging to genus Cladosporium is the most frequent and abundant regardless of latitude and height above sea level and above the ground present in the air. They mostly originate from agriculture environment.
- Need to exploit Fungal Volatile Organic Compounds (FVOCs) and merging them in **agroecological practices** by integrating them in actual agriculture.
- Exploration and Application of fungus like *Cladosporium sphaerospermum* strain TC09 in **Space based Research and Technology Development**
- Need to explore more **novel species** of *Cladosporium* with interesting biological and chemical diversity from environmental sources.
- A systematic bio-guided investigations are needed to explore the full potential and the diversity of **bioactive metabolites** produced by *Cladosporium sp.* offering an opportunity to discover novel natural products to benefit agriculture in an environmental friendly and sustainable manner.

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Dr. Anjana Bhatia (Head of Department Botany, Hans Raj Mahila Maha Vidhayla, Jalandhar, Punjab, INDIA)

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